AD NUMBER

AD836151

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Foreign Government Information; DEC 1963. Other requests shall be referred to Department of the Army Fort Detrick, Attn: Technical Release Branch [TID], Frederick, MD 21701.

AUTHORITY

SMUFD d/a ltr, 8 Feb 1972

TRANSLATION NO. 965

STUDIES OF THE EFFECTIVENESS OF AIR DISINFECTION BY MEANS OF ULTRAVIOLET RAYS

PART III

Surface Effect of Ultraviolet Rays

/Following is a translation of an article by Stanislaw Zdanicicki of the Military Institute for Hygiene and Epidemiology in the Polish-language periodical Przegląd Epidemiologiczny (Epidemiological Review), Vol. XVI, No. 3, Warsaw, 1962, pp 321-334/

The means which we use to disinfect air must act not only on the microorganisms carried in the air but also on objects, floor, etc (5, 12, 19). The mucus which comes out of the nose and throat when people cough or talk contains frequently numerous pathogenic microorganisms. These organisms fall on the floor, on the surface of objects, and when the water evaporates they come up in the air at the slightest commotion, depending on the size, and stay in the air for a shorter or longer period of time (2, 17). Some authors used special screens or saturated the floor with dust-absorbing fluids. In this way they prevented the microorganisms from rising and reduced their number in the air (8, 11, 16).

The purpose of this work was to demonstrate the action of ultraviolet rays, both reflected as well as direct rays, on microorganisms contained in the air.

In determining the conditions of the experiments, such as for example the time of irradiation, distance from the source of rays, type of lamp, etc, we used the data from the inquiry in the same way as we used them in the previous work.

Material and Methodology

Sources of Ultraviolet Rays

In these experiments we used sun lamps of the types used most frequently in hospitals, in the same way as we used them in studies concerning the effectiveness of ultraviolet rays with regard to microorganisms suspended in the air of a chamber.

Inoculation

In this test we used Staphylococcus aureus, which coagulates the blood corpuscles of sheep and rabbits, reacts positively to coagulating nitrogen and nitrogen phosphate, shows a strong tendency to form a fluff, and can be preserved with its biochemical characteristics in sugar. The microorganism resisted penicillin, streptomycin, chloromycetin, aureomycin, terramycin, erythromycin, and tetracyclin, and was slightly sensitive to neomycin. The inoculation element was obtained from the air of an operating room (Part III). The microorganisms were kept in a lyophilic form. We used in experiment a 24-hour culture of broth obtained from a lyophilic solution. We added 0.05% of Tween 80 to the broth. The optic density of the liquid culture was determined photometrically on a Visomat.

When we inoculated the plates with by methods A and B, we used an agar substance with blood. When we applied the seeding method C and D, we used as a rule agar solutions. After irradiation the plates were placed in a thermal cc. binet at at temperature of 37° for 24 hours.

Methods of Dissemination of Microorganisms

Method A. We used a pipette to pour 0.25 ml of liquid culture of microorganisms in the center of the plate. We covered the plate and made several circular movements to spread the culture evenly on the surface of the substance.

Method B. We used a pipette to pour 0.01 ml of fluid culture of microorganisms in the center of the plate. The liquid culture was diluted 1 : 1,000 by a physiological solution of salt, and we spread the solution by a glass rod (Drygalski spreader).

Method C. The microorganisms were disseminated by means of a special spreader which made it possible to inseminate 50 groups of the colony in parallel rows. The spreader consists of 50 glass rods with small balls at the end. The rods were fastened in a holder which makes it possible to keep the rod poised. During the dissemination the balls of the rods were kept on the surface of the nourishing substance only by their light weight and did not bring the microorganisms inside of the nourishing substance (figure 1, 2).

Method B. The microorganisms were disseminated in a similar manner as in Method A. We used the sprayer described above and disseminated the microorganisms on membrane filters (manufactured in USA) which were 70 m.m. in diameter and were used to catch microorganisms held in the air. An aseptic filter was placed in an aseptic petri plate, and we used the sprayer described above to disseminate microorganisms over it. After that the filter was transferred to another petri plate and after irradiation it was transferred to a plate with a solid substance in such a manner that the surface covered with microorganisms would be on the top.

Method of Direct Irradiation

Petri plates with solid substance or membrane filters were placed on saucers at an angle of 45° with regard to the base. The sun lamp was set up so that the rays would fall perpendicularly on the entire open petri plate. The plates were setup in ten rows, each row had 12 plates with serial numbers. The rows were 50 cm apart. The first row was placed 50 cm from the burner of the sun lamp. Plates No. 1, 2, 6, 9 were irradiated for ten minutes, No. 3, 5, 7, 10 - 30 minutes, 4, 8, 10, 12 more than 60 minutes. We also used irradiation lasting 100 minutes.

Method of Indirect Irradiation

A reflector of a sun lamp, placed 150 cm above the base, was turned perpendicularly to the ceiling. At a distance of 2 m from the lamp, we placed 12 plates at each level at a distance of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 m from the ceiling. The periods of irradiation were the same as those applied in the method of direct irradiation.

Humidity and Temperature

During the tests we determined the temperature and humidity of the room in which we irradiated the microorganisms.

We made the following checks during each experiment:

K. pow. - Designated the pollution of the air by microorganisms in the room in which the plates were irradiated. In the experiment we used the method of free settling of microorganisms by opening the plates for 10, 30, 60, 100 minutes. The plates were located around illuminated rows.

K. p. - Control of sterility of the support. Out of 100 plates which we prepared, we selected at random three plates and placed them in the thermal container at a temperature of 37° for 24 hours.

K - Control of dissemination of microorganisms. Plates covered with microorganisms without a previous irradiation were placed in the thermal container. When we took them out, we determined the increase of the number of microorganisms and compared it to the increase on the

irradiated plates. In each experiment we used five plates to control the dissemination.

No. 4 - Control on the effect of ultraviolet rays (Uv) on the plates which were used. We collected plates from the center and from the end of the rows which were not sown with microorganisms before irradiation (No. 1, 5, 12). Plates from the row No. 1 were taken after 10 minutes of irradiation, plates from the row No. 5 were taken after 30 minutes, and those from row No. 12 after 60 minutes of irradiation. After that the plates were sown with microorganisms, then taken out and compared with plates which were irradiated immediately after the dissemination.

The results of the experiments are given in the tables.

Table I

Direct Effect of Ultraviolet Rays on Microorganisms Disseminated on Solid Support (A Method of Dissemination)

Distance from Burner in Meters	Mark Pressure Lamps																							
	A Burner S-700 (new)												B Burner S-700 (used)											
	Time of Irradiation in minutes																							
	K	10	30	60	K	10	30	60	10	30	60	K	K	10	30	60	K	10	30	60	10	30	60	K
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
0.5	C	B	B	B	C	B	B	B	B	B	B	C	C	C	B	B	C	C	B	B	C	B	B	C
1.0	C	B	B	B	C	B	B	B	B	B	B	C	C	C	C	B	C	C	B	B	C	C	A	C
1.5	C	A	B	B	C	C	B	B	B	B	B	C	C	C	C	C	C	C	C	A	C	C	C	C
2.0	C	A	B	B	C	C	B	B	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
2.5	C	C	B	B	C	C	C	B	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
3.0	C	C	B	C	C	C	C	B	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
3.5	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
4.0	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
4.5	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
5.0	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C

Description of Results

Direct effect of ultraviolet rays on microorganisms disseminated on solid plates:

Ultraviolet rays reflected from the ceiling, which was covered with white glutinous paint, did not show any bactericidal effect when we used the dissemination methods A and C, regardless of the distance, time, and of the type of sun lamps. The growth of microorganisms on control plates was not different in terms of quantity or quality from the growth on irradiated plates.

Table I Continued

	High Pressure Lamp S-300 (near)												Low Pressure Lamp G 30 T 8 (near)											
	Time of Irradiation in Minutes																							
	K	10	30	60	K	10	30	60	10	30	60	K	K	10	30	60	K	10	30	60	10	30	60	K
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
0.5	C	C	B	B	C	C	B	B	C	A	A	C	C	B	B	B	C	B	B	B	B	B	B	C
1.0	C	C	B	B	C	C	B	B	C	C	A	C	C	B	B	B	C	B	B	B	B	B	B	C
1.5	C	C	C	A	C	C	A	B	C	C	C	C	C	B	B	B	C	B	B	B	B	B	B	C
2.0	C	C	C	C	C	C	C	A	C	C	C	C	C	B	B	B	C	B	B	B	B	B	B	C
2.5	C	C	C	C	C	C	C	C	C	C	C	C	C	B	B	C	C	B	B	B	B	B	C	C
3.0	C	C	C	C	C	C	C	C	C	C	C	C	C	C	B	B	C	A	B	B	A	B	B	C
3.5	C	C	C	C	C	C	C	C	C	C	C	C	C	C	A	A	C	C	A	B	C	C	B	C
4.0	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	A	C	C	B	C
4.5	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
5.0	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C

A - hemolysis covering up to 25% of the plate, B - 25 to 90%, C - over 90%. Optic density of the culture 0.05%. Relative humidity of the room 60-65%, temperature 19-20°.

K 1 - microorganisms disseminated after 10 minutes of irradiation of the plate, K 2 - after 30 minutes, K 3 - after 60 minutes.

Table I shows the direct effect of ultraviolet rays on microorganisms on a solid plate and on membrane filters.

Table I shows that the longer the period of irradiation, the greater the distance from the burner where we found that hemolysis was reduced. Also, there is a clear difference between the bactericidal effect of S-700 lamps (near) and that of S-700 lamps (used). Burner S-700 (used) gave the same amount of light as burner S-700 (near), but its bactericidal effect was much smaller. Low-pressure burner showed the highest degree of efficiency. The growth of microorganisms on plates K_1, K_3, K_{12} (effect of ultraviolet rays on the base) was not different from the growth on control plates which have been irradiated.

The pollution of the air in the room in which we carried out the tests amounted to several dozens of colonies which settled on the plate after the plate was opened for 60 minutes. There were no hemolytic microorganisms in the room colonies.

The S method of dissemination resulted in deviations of up to 300% on the controlled plates, and consequently we cannot comment on the results obtained after irradiation.

Table II

Direct Effect of Ultraviolet Rays on Microorganisms Disseminated on a Solid Plate (A Method of Dissemination)
Low Pressure Lamp, Philips S-700 (New)

Distance from Source in Meters	Time of Irradiation in Minutes: 10	30	60	Contr.	10	30	60	10	30	10	Contr.
	1	2	3	4	5	6	7	8	9	10	11	12
0,5	350	25	8	40	400	400	14	18	145	40	70	30
1,0	170	250	70	100	320	320	160	13	112	25	100	450
1,5	n	300	800	85	n	n	218	150	n	50	7	300
2,0	620	250	n	40	250	250	90	10	200	220	81	200
2,5	370	125	n	100	190	90	75	158	160	66	200	103
3,0	400	n	230	160	650	800	n	100	150	420	62	500
3,5	750	200	160	185	408	220	182	188	90	120	41	630
4,0	750	350	485	828	852	n	350	140	800	180	200	n
4,5	n	n	275	300	n	160	258	240	n	210	170	285
5,0	312	n	420	200	172	412	250	130	25	80	80	650

n - number could not be determined (in pcs)

Bacterial pollution of the air in the room: after 10 minutes - 4 colonies, after 30 minutes - 6 colonies, after 60 minutes - 1 colony

No hemolytic colonies found among gram colonies.
Control of dissemination of microorganisms: 218, 650, 420, 502, n (in pcs)
Control of effect on the plate (Kn).
Row 1 disseminated after 10 minutes of irradiation, row number 5 after 30 minutes, and row No. 12 after 60 minutes.
Optic density of liquid culture 0.05 Relative humidity of the room 65%, temperature 19°.

By using the C Method of dissemination, we could present the results in terms of percentages. In order to make the tables as clear as possible, we rounded up the percentages to 5 or 0. The bactericidal effect of the lamps increased in the following sequence: G 30, T 8, S-700 (new), S-300 (new), S-700 (used).

Fifty groups of colonies grew on each control plate.

Membrane filters were inseminated by a spreader of our own design. The results were rounded to 0 or 5 in the same way as in Table 3. We found out that the most effective ultraviolet rays were those produced by low-pressure lamps.

Table III

Direct Effect of Ultraviolet Rays on Microorganisms Disseminated on a Solid Base
(Method of Dissemination)

Number of Destroyed Groups of Microorganisms is Given in Per-Cent

Distance from Burner to Meters	A Burner S 700 (new)												B Burner S-700 (used)									
	Time of Irradiation																					
	K	10	30	60	K	10	30	60	10	30	60	K	K	10	30	60	K	10	30	60	10	30	60
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11
0,5	0	60	70	80	0	85	85	90	50	90	90	5	0	10	25	60	0	20	65	90	25	70	50
1,0	0	20	65	70	0	70	85	20	40	60	85	5	0	10	15	40	0	10	40	60	15	50	30
1,5	0	10	60	55	0	15	60	85	20	85	60	0	0	0	5	15	0	10	20	30	5	10	10
2,0	0	0	35	60	0	25	40	70	10	55	30	0	0	0	0	10	0	0	0	10	0	0	0
2,5	0	0	15	30	0	0	10	30	0	15	30	0	0	0	0	0	0	0	0	5	0	0	0
3,0	0	0	0	0	0	0	15	20	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0
3,5	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4,0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4,5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5,0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table III

Continued

Distance from Burner in Meters	High Pressure Lamp 3-200 (new)												Low Pressure Lamp G 30 U 3											
	Time of Irradiation																							
	K	10	30	60	K	10	30	60	10	30	60	K	K	10	30	60	K	10	30	60	10	30	60
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11
0,5	0	0	30	70	0	20	60	85	10	50	70	0	0	90	100	95	0	100	100	100	90	100	100
1,0	0	0	15	60	0	10	40	50	10	45	40	0	0	85	100	100	0	100	100	100	90	100	100
1,5	0	0	15	10	5	10	20	35	0	0	25	0	0	80	85	100	0	90	100	100	90	95	90
2,0	0	0	0	5	0	0	5	20	0	0	10	0	0	60	80	90	0	80	80	100	70	80	75
2,5	0	0	0	0	0	0	0	0	0	0	0	0	0	45	60	80	0	70	85	90	50	65	80
3,0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	15	80	0	50	70	85	25	60	15
3,5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50	0	30	65	70	0	20	0
4,0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30	0	0	15	50	0	0	0
4,5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	0	0	0
5,0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Cpld. density of fluid culture 0.0%.
Relative humidity of the room 55-65%, temperature 21-22°.

Table IV

Direct Effect of Ultraviolet Rays of Microorganisms Disseminated on Membrane Filters
(D Method of Dissemination)

Number of Destroyed Germs of Microorganisms Given in Per-Cent

Distance from Burner in Meters	High Pressure Lamp 0.700 (new) Time of Irradiation			High Pressure Lamp 0.700 (used) Time of Irradiation			High Pressure Lamp 0.700 (new) Time of Irradiation			Low Pressure Lamp 0.70 2.3 (new) Time of Irradiation		
	10 min.	30 min.	60 min.	10 min.	30 min.	60 min.	10 min.	30 min.	60 min.	10 min.	30 min.	60 min.
0,5	10	65	80	15	50	80	80	85	85	95	100	100
1,0	5	55	85	15	45	75	80	80	85	90	100	100
1,5	0	0	20	5	30	50	10	70	85	90	100	100
2,0	0	0	0	0	0	10	10	35	80	90	95	100
2,5	0	0	0	0	0	10	10	35	50	80	85	85
3,0	0	0	0	0	0	0	0	10	45	30	85	90
3,5	0	0	0	0	0	0	0	15	30	80	80	85
4,0	0	0	0	0	0	0	0	0	0	0	30	30
4,5	0	0	0	0	0	0	0	0	0	0	0	30
5,0	0	0	0	0	0	0	0	0	0	0	0	0

Optic density of cultures from 0.04 to 0.05
Humidity of the room between 55 and 65%, temperature 19° - 20°.

Table V

Direct Effect of Ultraviolet Rays on Microorganisms Disseminated on a Solid Base (C Method of Dissemination) During a Period of Three Hours

Number of Destroyed Colonies of Microorganisms Given in Per-Cent

Distance between Lamp and Plate in Meters	High Pressure Lamp S 700 (new)		Low Pressure Lamp G 30 T 8 (new)	
	Row I	Row II	Row I	Row III
0,5	100	100	100	100
1,0	100	100	100	100
1,5	100	100	100	100
2,0	100	100	100	100
2,5	95	95	100	100
3,0	100	100	100	95
3,5	100	100	95	90
4,0	100	100	60	65
4,5	90	90	60	65
5,0	90	90	50	60

Optic density of culture 0,2.
Humidity of the room 60-65%, temperature 21°.
Control of the effect of ultraviolet rays on the base: the plates, which were inoculated after 3 hours of irradiation do not show any difference from the control of dissemination of microorganisms.

The increase of the number of microorganisms on the control plates which have been irradiated for 180 minutes as well as on those which have not been irradiated did not show any difference in terms of quality or quantity.

When we irradiated the microorganisms with high-pressure sun lamps, the bactericidal effect of the rays emanating from the central part of the burner appeared to be clearly greater. This effect was described by some authors (9, 15).

Among the methods used to disseminate the microorganisms we found that we obtained the best results when we used a specially constructed spreader. When we used it, we could determine in terms of per-cent the number of groups of microorganisms which have been destroyed. Furthermore, by using the spreader which we mentioned above, we disseminated not only individual microorganisms but also their groups. In this way the experiment took place under conditions which were approximately those which exist under normal circumstances (1, 2, 4). By using the glass-rod spreader which disseminated the microorganisms in rows which were parallel and perpendicular to each other, we were able to avoid the possible errors due to pollution by microorganisms suspended in the air.

After the first few tests we abandoned the B Method of dissemination which showed deviations up to 300% in the amounts of control disseminations.

...k this method also in a test with E. coli. It is true that the deviations were much smaller (25-55%), but these tests were suitable either to confirm the results obtained by the irradiation of the plates with various types of sun lamps. In addition, by disseminating E.coli we obtained individual microorganisms which were much easier to kill than the conglomerates which are found under natural conditions (2).

Since the microorganisms reacted differently to ultraviolet rays used in the tests, we used the same microorganisms which we used in the previous work (when we studied the effects of radiation on microorganisms suspended in the air of a chamber). It was a microorganism which represents best danger in hospital sections for infectious diseases (6, 7, 13, 14, 18, 22, 23, 25). During the tests we did not observe any toxic characteristics of the plates which were subjected to irradiation. The growth of microorganisms on irradiated plates was not different from the growth on control plates which have not been subjected to irradiations. Hollaender (10) states that the toxic properties of the plates appear only after long irradiation involving much greater doses of ultraviolet rays than those which we used in the tests.

Other authors (3, 9, 21) who irradiated microorganisms which have been disseminated on a solid bed did not notice any toxic effect on the microorganisms even after ultraviolet radiation which lasted for six hours. In addition to the lack of any toxic effect of the irradiation of the experimental bed, the proportion of microorganisms which were killed was the same as the per-cent obtained on membrane filters. We used the sedimentation method in those tests, which is rather obsolete with regard to the determination of the pollution of the air. However, it was certainly proper to use that method, because we were not interested in the actual pollution of the air but rather in the amount of conglomerates which fell on the tables.

Before and after each test we washed the tables and beds in the test room by a 0.5% solution of chloramine. The lamps which we used during the tests were connected to a voltage stabilizer, so that we could get systematically a no-load current. When we irradiated microorganisms in a ball-shaped container, we found live microorganisms on the inside of the container about 80 cm from the burner. Microorganisms disseminated on the bed or on the membrane filter, which were irradiated for a shorter period of time, were killed even when they were 2 to 3 times as far from the burner. This can be explained by the concentrated effect of the reflectors of the sun lamps. We removed the reflectors from the sun lamps when we carried out the tests in a chamber, so that the irradiation would be even. When we irradiated microorganisms which have been disseminated on the surface or on membrane filters, we used sun lamps with reflectors. Low-pressure lamps, which are used primarily for therapeutic purposes, are equipped with reflectors which focus the rays on a certain point. Low-pressure lamps which are used to kill bacteria do not have such reflectors. The protocol of research work carried out by the Chair of Radiology of the Warsaw Polytechnical School shows that the bacteriocidal

some 2537 Å was 8 to 12 times wider, depending on the length, when we used a focus reflector (20). The results which we obtained with regard to the effect of ultraviolet radiation on microorganisms located on the surface were not different from the results obtained by other authors. Bau H (21) used equipment consisting of 5 low-pressure lamps (without reflectors). He succeeded to destroy the microorganisms which were disseminated on an agar bed only after he irradiated them for several hours. Other authors obtained similar results (9, 24).

Summary

1. Regardless of the type of lamps which we used and the period of irradiation, indirect ultraviolet radiation did not show any bactericidal effect with regard to microorganisms located on the surface of an agar bed or on membrane filters.

2. Regardless of the methods used to disseminate the microorganisms, a low-pressure lamp generally showed a greater bactericidal effect than a high-pressure lamp.

3. A used lamp S-700, which produced the same amount of light as a new lamp S-700, showed a considerably smaller bactericidal effect.

4. By using a special spreader we were able to disseminate groups of microorganisms and make a per-cent comparison of the bactericidal effect on microorganisms which were disseminated on solid beds and on membrane filters.

Conclusions

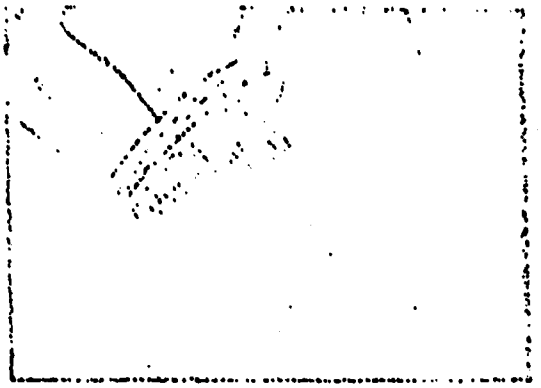
On the basis of the results of our inquiry concerning air disinfection by means of ultraviolet rays in hospitals (see Part I) and on the basis of the results of research concerning the effect of ultraviolet rays on microorganisms suspended in the air (see Part II) and not on the surface, we find that it is necessary to do the following:

1. The inappropriate high-pressure lamps should be replaced by bactericidal low-pressure lamps.

2. We should prepare instructions concerning ultraviolet irradiation of the air in hospital rooms.

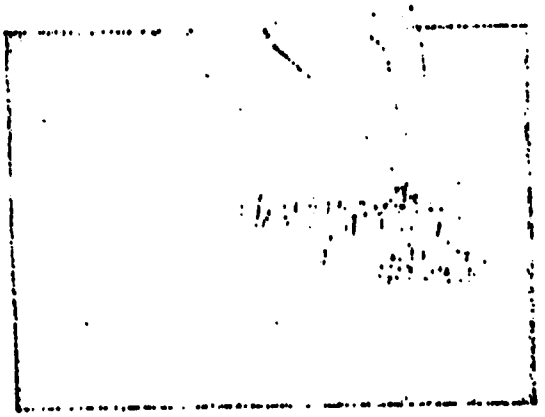
3. We should make it obligatory to make a periodical study of the air.

The author expresses heartfelt thanks to Prof. Dr. J. Kostrzewski for valuable suggestions and observations concerning the above work.



Rye. 1

Figure 1.



Rye. 2

Figure 2.